Performance and yield stability of doubled haploid population of wheat (*Triticum aestivum* L.) under high temperature regime

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Abstract

India, the second most populated country and the largest wheat producer worldwide, is vulnerable to global warming especially heat stress. In the present investigation, the doubled haploid population derived from PBW343/IC252478 cross was characterized for various phenotypic and morpho-physiological traits and subjected to stability analysis under heat stress conditions. These lines were planted on a single location i.e., Agricultural farm of Rajendra Prasad Central Agricultural University, India for two successive seasons 2017/2018 and, 2018/2019 under three different sowing dates (Controlled or timely, late and, very late sown conditions). Here, the location preferred for this study was because it represents a hotspot for wheat production and the major constraint for the wheat grower is inclining heat stress. The alpha lattice design was used for the current investigation with three replicates. The overall objective of this study was to identify the ideal double haploid lines for heat-stressed conditions. The results revealed that heat stress had a significant adverse impact on all considered traits contributed to overall yield losses of about 50%. Stability measurements, and genotype ×environment interaction (GGE), were useful tools to determine the ideal lines for late sowing (heat stressed condition) and very late sowing condition (terminal heat stress). Therefore, in the ranking of genotypes for both mean yield and stability performance across the six environments, DH 71, DH 150, DH 64, DH 138, DH 98, DH 84, DH 62, DH 104, DH 74, DH 3, DH 104, DH 107 & DH 156 were ranked closest to ideal genotype, these were highly adapted, most stable, heat tolerant and high yielding lines indicating them as the most desirable genotypes out of 167 lines studied. Hence, the physiological traits SPAD index (Soil plant analysis development) and Canopy temperature (CT) can be used effectively to screen out the line for heat tolerance. In addition, the stable wheat genotypes identified could be used in the future wheat breeding programs.

.***Key Words:*** Soil plant analysis development; Canopy temperature; Heat stress and Double haploid

Introduction

Climate change threatens to exacerbate existing threats to food security and livelihoods due to a combination of factors that include the increasing frequency and intensity of climate hazards, diminishing agricultural yields and reduced production in vulnerable regions, rising health and sanitation risks, increasing water scarcity, and intensifying conflicts over scarce resources, which would lead to new humanitarian crises as well as increasing displacement (IPCC, 2007). The most important climate change is rising temperature. Wheat (*Triticum aestivum* L.) is the second most consuming cereal worldwide. This crop is too much vulnerable to biotic and abiotic stresses. While considering Indo-Gangetic plains, heat stress widely affects wheat production. By 2050, the global average temperature is projected to have risen between 2 and 4 °C above the pre-industrial climate. Empirical evidence suggests that increases in temperature in the period 1980–2008 have already resulted in average global wheat yield reductions up to 5.5%, compared to a non-climate scenario (Lobell et al., 2011).

A breeder needs a handy tool to identify their variety. Measuring canopy temperature is a suitable tool to serve the purpose. In the case of wheat, breeding for heat tolerance is an important aspect. The relationship between Canopy Temperature (CT) and grain yield under heat stress conditions is well established (Olivares-Villegas et al. 2007; Lopes and Reynolds 2010; Lopes et al. 2012). Cooler canopy temperature (CT) is associated with heat tolerance (Pinto et al. 2010). Stay-green in the post-anthesis phase is reported to be associated with heat tolerance in several crops (Borrel et al. 2000; Campos et al. 2004) and is utilized for breeding heat-tolerant cultivars in wheat (Christopher et al. 2015).

Yield is a complex quantitative trait and is greatly influenced by the external environment, which results in a scale or rank shift in its performance (Dia, 2012; Dia et al., 2016a). This relative shift of genotype performance from one environment (multilocation or multiyear) to another is known as genotype × environment interaction (G×E) (Dia et al., 2012a; Dia et al., 2012b). The multi-location trial has several benefits, including the ability to select genotypes that are widely adapted based on the average yield performance in various environments, the ability to select genotypes that are specifically adapted to certain environments, and the ability to identify redundant test locations or environments such that information about genotypes is obtained with minimal duplication (Yan *et al*., 2010). GGE biplot (genotype main effect plus genotype x environment interaction) was found useful for the analysis of GE interactions (Ahmadi et al. 2012; Butron et al. 2004; Farias et al. 2016; Karimizadeh et al. 2013; Laffont et al. 2007; Samonte et al. 2005). Increases in local temperatures can generate devastating agricultural losses and can be critical if they coincide with key stages of crop development (Wollenweber et al., 2003). The yields of many cereal crops can be drastically reduced by temperatures above 32 °C during the flowering stage: for instance, rice grain sterility occurs in temperatures in the mid–30s (Vara Prasad et al., 2003 and Hatfield et al., 2011). Empirical studies using historical crop-trial data have predicted that the adverse impact of heat stress on crop yield can be mitigated by shifting sowing dates (Dhillon and Ortiz-Monasterio, 1993). Substantial studies have been conducted to identify high-yielding and consistent performing genotypes (also known as stable genotypes). However, most of the high stable genotypes are less predictable across shifted sowing dates since plant breeders often perform analysis of two-way data (G×E) for several consecutive years to detect stable genotypes without taking crop management practices into account (Dia et al., 2009; Gathala et al., 2011; Sapkota et al., 2017; Weindorf et al., 2008a; Weindorf et al., 2008b). In this paper, we propose to identify high-yielding and stable wheat lines across the considered location and over three different sowing dates or six environments.

Materials and methods

Plant Materials and Field experiment

A mapping population consisting of 167 doubled haploid lines developed by making crosses between two parental genotypes, PBW343 (heat susceptible) and IC252874 (heat tolerant). Development of the DHs was done in the Washington State University, Pullman, USA, and evaluation was carried out at the research farm of the Rajendra Prasad Central Agricultural University, Pusa, Bihar, India (25° 57′ 08″ N; 85° 40′ 13″ E). An offseason facility at the Research Station, Punjab Agricultural University, Keylong, Himachal Pradesh, India, was used for advancing the generations. Two experiments were carried out in 2017–2018 and 2018–2019 growing seasons. In each trial, 167 doubled haploid lines and their parents (PBW343 & IC252874) were planted in alpha lattice design with three replications. Experimental plots consisted of two 2 m long beds spaced at 60 cm and comprising four rows per bed. Proper agronomic practices and irrigation were provided to avoid yield reduction during the crop cycle. The experiment was done on three different dates of sowing viz. November, December, and January with twenty-five days of the gap. The experiments with delayed sowing on January 23 were considered as terminal heat stress in both subsequent growing seasons.

Variables measured

Data were recorded for days to heading, maturity was measured with 75% yellowness. Plant height was measured on a random sample of five plants in each plot as the distance from the soil surface to the tip of the spike awns was excluded at harvest time (cm). Total leaf chlorophyll content (SPAD index) was estimated using a spad-502 chlorophyll meter (spad-502 plus, Konica Minolta, Kearney, NE, USA), during the flowering stage. Canopy temperatures were measured using a handheld infrared thermometer (KM 843, Comark Ltd., Hertfordshire, UK) with a field view of 100 mm to 1000 mm. Canopy temperatures data were taken from the same side of each plot at a 1m distance from the edge and approximately 50 cm above the canopy. Plants at the center of each plot were selected to count the number of tillers at the growth stage (GS85). Readings were made between 1300 and 1500 h on sunny days. Thousand-grain weight (TGW in g) and grain yield (GY in g) was calculated by weighing grains of harvested plants from an area of 2 m^2 in each plot excluding border effect (Sayre et al. 1997).

Statistical analysis

Analysis of variance (ANOVA) showed that genotypes were significantly different for heat tolerance for all the traits during both years. Pearson correlation coefficient between the traits for the trials was performed using GenStat-12.1/2009 (http://www.vsni.co.uk/genstat) program. The following stability measurements were performed on thousand-grain weight and grain yield under the six environments (three seasons and two years which composes six environments), i.e., coefficient of variability (CVi) [Pinthus 1973], regression coefficient (bi) [Eberhart 1966], Wricke's ecovalance (Wi) [Mulusew et al., 2014], superiority measure (Pi) [Lin et al., 1988], Perkins and Jinks (Di) [Blanco et al., 2012], and average absolute rank difference of genotype on the environment. (Si)[Akcura et al., 2008]. To determine the effects of GEI on grain yield, the yield data recorded from all three environments (E1, E2, and E3) were subjected to AMMI and GGE biplot analysis using Gen Stat 14th edition (VSN International, Ltd, Hemel Hempstead, UK) and R software 3.3.0 respectively. Then GGE biplots were used to graphically show the genotypes and environments (Yan et al., 2001). Angles between environment vectors were used to judge correlations (similarities/ dissimilarities) between pairs of environments (Yan and Kang 2003).

Result and Discussion

Analysis of Variance

The analysis of variance for heading, maturity, plant height, peduncle length, total leaf chlorophyll content (SPAD index), canopy temperatures, tiller no., biomass, thousand-grain weight, and grain yield are presented in **Table 1.** The results indicated a highly significant effect (p-value < 0.01) for the six environments (three growing seasons on one location which composes six environments) on all traits. Furthermore, sowing dates had a highly significant effect on all traits. More importantly, the analysis of variance revealed highly significant variance among the double haploid lines. Our results suggested that the magnitude of differences among lines was sufficient to provide a scope to characterize the effect of terminal heat stress (very late sown condition). The Pearson correlations coefficients among the studied traits are presented in **Fig 1**

Phenotypic assessment of double haploid lines

The mean performance of double haploid lines showed each lines, were differing among themselves with regard to various considered characters for different date of sowing. Mean values of the double haploid lines for the characters viz. Days to heading for six environments are as follows 71.45, 69.08, 67.86, 65.69, 58.50 and 54.88, days to maturity 109.20, 106.97, 105.73, 103.16, 95.39 and 91.77, plant height 98.07, 98.27, 94.46, 95.06, 87.10 and 83.54 cm, peduncle length 37.50, 37.93, 34.27, 37.68, 30.22 and 26.75 cm, tiller no. 159.87, 156.2, 150.13, 152.75, 148.54 and 141.5, canopy temperature 34.28, 30.52, 37.34, 33.55, 42.62 and 38.86 ℃, chlorophyll content (SPAD index) 41.14, 37.35, 34.42, 30.72, 27.85 and 24.09℃, thousand grain weight 34.75, 31.06, 27.32, 28.87, 25.65 and 22.01 gm, grain yield 142.47, 138.92, 100.86, 133.27, 126.61 and 98.43 gm and biomass 473.79, 470.36, 469.98, 466.58, 462.43 and 458.80 gm respectively. DH 71 (Env -1, 2 & 3) & DH 150 (Env-4, 5 & 6) showed most extended days to heading and DH 160 (Env-1, 2, 3,4, 5 & 6) was found to be the decreased in days to heading. DH 80 (Env- 1), DH 64 (Env- 2, 3, 4 & 5) & DH 138 (Env- 6), showed most extended days to maturity for the environments while DH 160 (Env- 1, 2, 3, 4 & 5) & DH 150 (Env- 6) showed most shortened days to maturity for the environments. DH 98 (Env- 1 & 3), DH 45 (Env- 2), DH 154 (Env- 4) & DH 84 (Env- 5 & 6) was found to be the tallest among all the lines and DH 117 (Env- 1, 5 & 6) & DH 159 (Env- 2, 3 & 4) was found to be the dwarfish lines. DH 97 was consistently found to be the tallest and DH 128 has dwarfish peduncle length under all the environmental conditions viz. (Env-1, 2, 3, 4, 5, & 6). DH 155 has having the highest and DH 14 has the lowest no. of tillers under all the environmental conditions. DH 104 has having the highest canopy temperature in all the environmental conditions but in case of lowest canopy temperature, DH 157 was found minimal in two subsequent environments which is (Env- 1 & 2) and DH 62 was lowest one in (Env-3). However, In case of (Env- 4, 5 & 6), DH 150 has the lowest canopy temperature. Under the environmental regime, (Env-1, 2 & 3), DH 104 has the highest chlorophyll content (SPAD index) and DH 157 was found to be the lowest in (Env- 1& 2).

However, DH 150 was the lowest one in (Env-3). In case of (Env- 4, 5 & 6), DH 113 & 107 was found to be the highest and DH 63 has the lowest SPAD index. DH 113 (Env-1) & DH 104 (Env-2, 3, 4, 5 & 6) showed the maximum thousand grain weight and DH 62 (Env-1), DH 79 (Env-2 & 3) & DH 156 (Env-4, 5 & 6) showed minimal thousand grain weight among the lines. DH 22 (Env-1), DH 104 (Env-2, 3 & 4) & DH 3(Env-5 & 6) showed the maximum grain yield while DH 156(Env-1, 2 & 5) & DH 61 (Env-3, 4 & 6) showed lowest grain yield. DH 145 (Env-1, 2, 3, 4, 5& 6) has the maximum biomass across all the environment and DH 74 (Env-1, 2, 3 & 4) & DH 156 (Env- 5& 6) was the least of all.

Interrelationships among the Studied Traits under the three different Sown Conditions

Results of the study have reflected the effect of sowing date on the response of the doubled haploid lines for the heading, maturity, plant height, peduncle length, Total leaf chlorophyll content (SPAD index), Canopy temperatures, tiller no., biomass, Thousand-grain weight, and grain yield respectively. The adverse effect of heat stress can be seen on the DH lines. In this study, we have assumed environments 1 & 4 as (controlled condition) and environments 2 & 5 (late sown condition) and 3 & 6 (Very late sown condition). These different environmental conditions have provided a clear picture for a better understanding of heat stress or terminal heat stress regime regarding DH lines. Here, the pooled mean data of 2016-17 & 2017-18 have shown the reduction of phenotypic and increment in the morpho-physiological measurement's across the environments due to heat stress. The late sown condition had a significant adverse effect on all the studied characters. In the late sown condition, the days to heading shortened from 65.69 to 54.88 days. In the same manner, the late sown condition shortened the days to maturity from 103.16 to 91.77 days. The late sown condition has an adverse impact on plant height in which the mean height across lines was dropped from 95.06 to 83.54 cm. Similarly, peduncle length reduced from 37.68 to 26.75 cm. Moreover, the late sown condition decreased no. of tillers from 152.75 to 140.51and decreased biomass from 466.58 to 432.7. Furthermore, the late sown condition increased canopy temperature from 33.55 to 45.09 ℃ and chlorophyll content (SPAD index) decreased from 30.72 to 19.43 ℃. Ultimately, the thousand-grain weight reduced from 27.67 to 18.34 gm followed by the grain yield reduction from 97.26 to 65.04 gm. Among the DH lines, under different sown conditions, DH 71, DH 150, DH 64, DH 138, DH 98, DH 84, DH 62, DH 104, DH 74, DH 3, DH 104, DH 107 & DH 156 were found to be resistant lines. Whereas DH 160, DH 145, DH 113 & DH 22 lines were susceptible to heat stress, and DH 117, DH 159, DH 45 & DH 157 were found to be moderate towards heat stress conditions.

Genotype × Environment Interaction (G × E) for thousand grain weight and grain yield

Based on the mean performance of double haploid lines, the subset consisting of 167 lines, which represented the whole variation in grain trait viz. thousand-grain weight and grain yield for heat tolerance, from which 30 high-performing lines were selected for further study. Two characters viz. thousand-grain weight and grain yield, considered for the stability study, are a quantitative and complex trait that was found to be responsive to double haploid lines by environment interaction (G × E). Additionally, grain yield followed by thousand-grain weight is the most critical parameter that determines a cultivar's acceptance by growers. The results of the stability parameters used in this study are presented in **Table 2(a&b)**. Under the combined sowing dates (six environments) model, i.e., three sowing dates, one location, and two years. For the trait thousand grain weight under controlled condition, DH 113 was the most stable cultivar in two measurements coefficient of variation (C.V%) & Superiority measure (Pi), DH 22 in Wrike's ecovalence (Wi), DH 104 in Regression coefficient (bi), and DH 201 in Perkins and Jinks (Di). Whereas, Under the late sown condition, DH 107 was the most stable cultivar in measurements such as coefficient of variation (C.V%), DH 113 Superiority measure (Pi), DH 22 in Wrike's ecovalence (Wi), DH 104 in Regression coefficient (bi), and DH 201 in Perkins and Jinks (Di). Under the very late sown condition, DH 113 was the most stable cultivar in measurement coefficient of variation (C.V%), DH 104 in Superiority measure (Pi) and DH 65 in Wrike's ecovalence (Wi) & Perkins and Jinks (Di), and DH 126 in Regression coefficient (bi). Moreover, for the trait grain yield under controlled condition, DH 3 was the most stable cultivar in two measurements coefficient of variation (C.V%) & Regression coefficient (bi), DH 104 in Superiority measure (Pi), DH 102 in Wrike's ecovalence (Wi), and DH 113 in Perkins and Jinks (Di). Under the late sown condition, DH 3 was the most stable cultivar in two measurements coefficient of variation (C.V%) & Regression coefficient (bi), DH 104 in Superiority measure (Pi), DH 102 in Wrike's ecovalence

(Wi), and DH 107 in Perkins and Jinks (Di). Under the very late sown condition, DH 3 was the most stable cultivar in two measurements coefficient of variation (C.V%) & Regression coefficient (bi), DH 104 in Superiority measure (Pi), DH 126 in Wrike's ecovalence (Wi), and DH 35 in Perkins and Jinks (Di). The DH lines adaptability and good performance or stability were also provided with the help of plot CV %. For the trait thousand grain weight, under controlled condition, DH 5, 31, 34, 38, 45, 48, 57, 59, 63, 68, 71, 76, 81, 108, 117, 121, 130, 134 and, 166 showed adaptability and rest of the lines showed stable performance. Under the late sown condition, DH 65, 73, 80, 91, 127, 129, 136, 152 and, 201 were found to be adaptable lines and the rest of the lines showed stable performance. Moreover, under very late sown condition, DH 12, 18, 21, 40, 48, 63, 67, 69, 92, 97, 99, 132, 154, 156, and 174 were adaptable lines and the rest of the lines showed stable performance. **(Fig2).** For the trait grain yield , under controlled condition, DH 2, 5, 10, 41, 68, 70, 76, 80, 94, 107, 113, 115,118, 120, 145, 146, 149, 170,188, 195, 197, 201 and 202 were found to be the adaptable lines and rest of the lines showed stable performance. Whereas, under late sown condition, DH 2, 94, and 188 were found to be adaptable lines and the rest of the lines showed stable performance. Moreover, under very late sown condition, DH 2, 5, 10, 41, 53, 70, 76, 80, 94, 96, 107, 120, 146, 155, 161 and, 188 showed adaptability, and the rest of the lines showed stable performance. **(Fig3).**

Polygon view of GGE biplot for thousand grain weight and grain yield

The biplot analysis conducted on the double haploid lines indicated a variable response under the three different dates of sowing or environmental conditions for two years. The biplots were generated; for controlled, late sown, and very late sown conditions. The polygon was formed by connecting the DH lines that were further away from the biplot origin, such that all other DH lines were contained in the polygon. The DH lines located on the vertices of the polygon performed either the best or the poorest in one or more locations since they had the longest distance from the origin of the biplot. The 'polygon' (which-won-where) view of the GGE biplot divides the biplot into sectors via perpendicular lines (rays) passing from the polygon sides. The polygon is drawn by joining extreme genotypes of the biplot. If environments fall into different sectors, then different genotypes won in different sectors, and a crossover G×E pattern exists. The winning genotype for an environment or set of environments in a sector is the vertex genotype. Conversely, if all environments fall into a single sector, a single genotype had the highest yield in all environments. The vertex genotype in a sector where no environment is present is considered to be a poor performer in all test environments. Genotypes within the polygon were less responsive to location than the vertex genotypes. For the trait, thousand-grain weight (2016-17 or Y1), the vertex double haploid lines in the GGE biplot (combined sowing dates or D1, D2, D3) were DH 146, 195, 60, 202, 65, 155, 124, 5, 201, 169, 15 and 170. In the subsequent year (2017-18 or Y2) for thousand-grain weight, the vertex double haploid lines in the GGE biplot (combined sowing dates or D1, D2, D3) were DH 5, 125, 169, 146, 170, 195, 60, 155 and, 124(**Fig 4**). For the trait, grain yield (2016-17, Y1), under the three different sowing dates (D1, D2, D3), the vertex DH lines were DH 201, 195, 142, 90, 2, 145, 107, 35, 164, 18, 223, 104 and 3. In the following year (2017-18 or Y2) for grain yield, the vertex double haploid lines in the GGE biplot (combined sowing dates or D1, D2, D3) were DH 201, 170, 195, 145, 107, 18, 22, 113, 104 and 3(**Fig 5**). Interestingly, the highest actual and predicted yielding lines are almost the same across environments. This suggests that different DH lines won in different sectors, and a crossover G×E pattern existed.

Mean vs. stability view of GGE biplot

The 'average environment coordinate' (AEC) view based on genotype-focused singular value partitioning can be referred to as the 'mean vs. stability' view (Yan et al., 2007) of GGE biplot. That view facilitates genotype comparisons based on mean performance and stability across environments. The most stable genotype was located almost on the AEC abscissa (horizontal axis) and had a near-zero projection onto the AEC (vertical axis). A longer projection to the green line, regardless of the direction, represents a greater tendency of entry by tester interaction for the entry, which means more variable and less stable across tester. Based on their mean performance across all testers, the entries are ranked along with the AEC. For grain yield **(Fig 6)** and thousand-grain weight **(Fig 7)**, it is depicted in the respective biplot. Overall, the pattern of lines stability varied across environments for the studied location.

Discussion

Wheat (*Triticum aestivum* L.) is one of the world's largest cereal crops and the most important staple food of about two billion people (36% of the world population). Globally, 20% of the food calories consumed and 55% of the carbohydrates are provided by wheat (Breiman and Graur 1995). It is a cool-season crop that is found to be sensitive to heat stress during the reproductive stage [Catro et al.,2007]. Heat stress tolerance is a complicated process controlled by several small effect genes or QTLs and is often confounded by differences in plant morphology and physiology under different environments [Mohammed et al., 2009]. Heat stress tolerance and maintaining high grain yield under heat stress are considered as one of the most critical aspects of wheat improvement [Cossani et al., 2012]. The double haploid lines used in the study were genetically established to be grown in environments of Indo-Gangetic plains under late sowing dates, where the temperature is inconsistent. Multilocation trials are usually practiced by breeders for selection. The current investigation shows the effect of heat stress on several phenotypic and morpho-physiological traits. The elevated temperature during the late sown condition decreased days to heading, maturity, plant height, peduncle length, total chlorophyll content, tiller no., biomass, thousand-grain weight followed by grain yield and on the other hand canopy temperature is increased with the increasing terminal temperature. Due to structural damage with respect to heat stress, the total chlorophyll content reduced significantly under the late sown condition, [Chen et al., 2012]. However, the current study also showed a similar pattern in the case of chlorophyll content and the reason behind the strong correlation between total chlorophyll content and yield might be the different growth stages used for the measurement of chlorophyll content. Previous reports suggested that the plants start losing chlorophyll content once it achieves grain filling stage [Del et al., 1998]. Due to the increasing temperature (late sown and very late sown conditions), canopy temperature also increased among all the doubled haploid lines. The correlation between canopy temperature and grain yield was negative and significant which is similar to previous reports [Asthir et al., 2015]. The current study indicated variable response of the considered lines to heat stress across all the traits under different environmental conditions and it is similar to previous reports (Khan et al., 2015) Therefore, understanding the specific impacts of shifted sowing dates and elevated temperature on wheat yield stability in Indo-Gangetic plains is important. Based on normal (November), late (December), and very late (January) sowing dates, several lines showed stable performance. The stable and adaptable performing lines also changed as the date of sowing shifted from November to January. In the current study, several lines outperformed under late and very late sown conditions (heat-stressed conditions) while some of the lines performed average under (heat-stressed conditions). Previous results imply that the lines used in this study contain different combinations of genes governing their response to sowing dates and tolerance to heat stress, and this result agrees with previous findings. As the current scenario suggests, the Genotype–environment (G × E) interaction is a kind of reliable method but at the same time quite difficult for the screening of lines due to the performance of lines or genotypes gives different responses under multi-location or multi-year trials. The most vital character from the grower's perspective is grain yield, which they solely rely upon to some extent. Grain yield is a polygenic trait that is complex in nature and it has a larger impact on G × E interactions. Therefore, stability measurement became an important process for breeders before release of their variety. This method provides the privilege to select superior and stable lines over mean performance. In the current investigation, we have used five stability parameters considering the thousand-grain weight and grain yield, which were not enough in revealing ideal lines for three different dates of sowing, which is similar to previous reports [Abbas et al., 2013]. This might be caused due to the statistical methods that the stability parameters rely on [Witcombe et a;., 1988]. In our study, the GGE biplot revealed the most stable lines across the environments. It is presumed that the most stable lines are those which are high-yielding in a wide range of environments. In the current study, several lines showed well performance under controlled conditions but not under the late sown and very late sown conditions (heat-stressed conditions) and vice-versa. While some of the lines performed average under heat-stressed conditions. This study was conducted to screen and evaluate the double haploid lines for the considered characters especially thousand-grain weight and grain yield were estimated to check the effect of heat stress under a late sowing regime. Several lines were selected as stable performers under stress conditions. Therefore, On the basis of this preliminary investigation, QTL mapping of these double haploid lines is also under progress considering morpho-physiological traits contributing to heat tolerance followed by marker-assisted selection.

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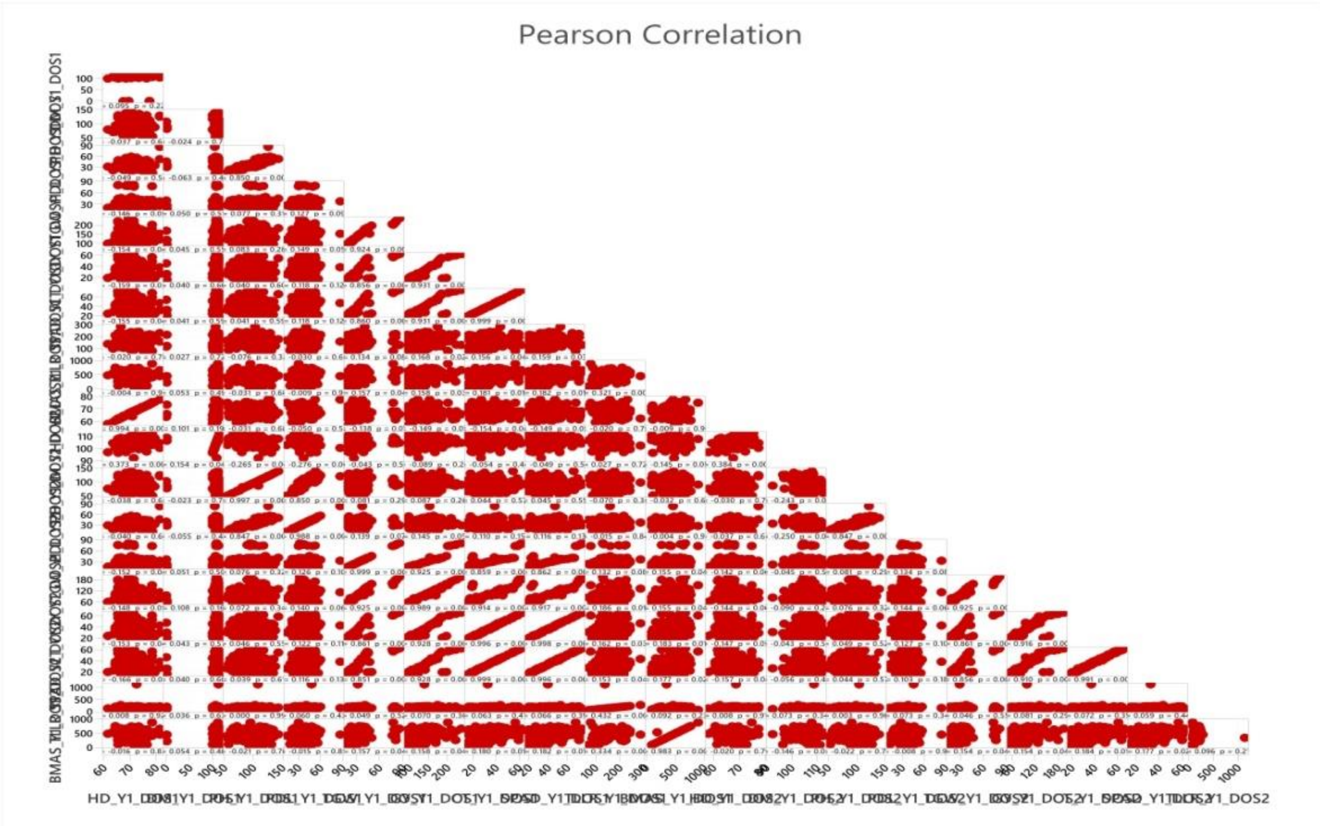
Source of Variation	df	DHE	DM	PHE	TGW	GY	CT	SPAD	BMAS
Replications	3	36.04**	40.5**	0.0087**	10.60	12483.77	2.64*	9.7**	4120**
Year	2	8.6*	854.1**	639.9**	3553.56**	1476872.00**	3770.45**	7047.3**	428
Line	166	72.6**	40.5**	0.0143*	33.59**	293175.25**	1.75**	2.8**	350
Year X Line	322	24.1**	19.0**	76.8**	23.10**	278252.75**	1.55**	1.97	6513**
Error	762	14.27	33.09*	4.179	11.10	157771.44**	0.73	0.84	110.98*
Heritability		0.67	0.53	0.50	0.67	0.72	0.70	0.68	0.50

Table 1: Pooled Analysis of variance for different morpho-physiological and yield traits

Fig 1: Phenotypic correlation between all the traits measured in the doubled haploid population PBW343/IC252874



	TGW (Nov)					TGW (Dec)					TGW (Jan)				
GEN	**CV(%)**	**bi**	**DJi**	**Wi**	**Pi**	**CV(%)**	**bi**	**DJi**	**Wi**	**Pi**	**CV(%)**	**bi**	**DJi**	**Wi**	**Pi**
201	11.6496	1.0693	0.0193	0.4344	923.3169	10.8357	1.0538	0.0017	0.0867	897.0461	11.7616	1.0936	0.0513	0.2789	949.5877
170	10.373	0.9804	0.2738	1.1235	867.4196	9.1831	0.9184	0.0574	0.2533	850.4157	11.2125	1.0796	0.64	0.8043	884.4234
195	11.689	1.0836	0.0782	0.832	906.5165	11.355	1.1181	0.0291	0.4394	877.2023	10.9668	1.0273	0.1557	0.1749	935.8307
65	10.619	1.0709	0.0244	0.4718	779.264	10.2426	1.0906	0.0115	0.2527	753.6329	10.1255	1.0386	0.0441	0.0828	804.8951
145	5.4722	1.0079	0.079	0.3208	26.7699	5.4791	1.0426	0.0287	0.082	23.3631	4.96	0.9514	0.1588	0.22	30.1767
125	8.0148	0.9137	0.0988	0.9486	587.6541	7.9843	0.9523	0.0338	0.1007	574.0159	7.2644	0.851	0.2027	0.7791	601.2923
35	4.8467	0.9104	0.0479	0.7876	17.5561	4.8473	0.9375	0.0182	0.1331	15.7494	4.4125	0.8662	0.0945	0.5588	19.3628
164	5.3947	1.0082	0.0838	0.3401	19.7658	5.408	1.0439	0.0301	0.0867	16.9582	4.8803	0.9501	0.1689	0.2335	22.5734
18	5.687	1.083	0.0691	0.7889	12.7635	5.6674	1.1155	0.0263	0.4189	10.2729	5.1961	1.0299	0.1367	0.1599	15.254
22	5.0294	0.9701	0.0577	0.2974	8.2597	5.0297	0.9998	0.0218	0.0218	6.9454	4.5764	0.9216	0.1143	0.2738	9.574
113	4.6411	0.9117	0.0656	0.8417	3.6732	4.6658	0.9433	0.0238	0.1183	3.1838	4.1882	0.8603	0.132	0.6387	4.1626
104	6.3316	-0.2198	28.1799	223.3256	14.3008	8.668	-1.103	49.3098	179.3804	28.6017	4.6839	1.0007	0.0919	0.0919	5.945
3	11.8148	1.0385	0.0745	0.4081	986.911	11.4764	1.0722	0.0275	0.1808	959.7711	11.0815	0.9835	0.1487	0.1557	1014.051
94	9.6758	0.9077	0.0916	0.9994	886.0155	9.5566	0.9449	0.0316	0.1209	869.651	8.8599	0.8473	0.1873	0.7929	902.3799
155	12.5993	1.1293	0.05	1.4433	958.8924	12.114	1.1571	0.0205	0.7467	925.5818	12.0114	1.0838	0.0959	0.2781	992.2029
102	11.1548	1.0386	0.0768	0.4181	899.4326	10.8717	1.0728	0.0283	0.1843	873.528	10.4203	0.9829	0.1535	0.1611	925.3371
168	11.5767	1.0668	0.0794	0.6496	915.3384	11.2585	1.1016	0.0293	0.333	887.0973	10.8427	1.0101	0.1585	0.1612	943.5795
149	10.0372	1.0078	0.0767	0.3114	783.6817	9.8396	1.0419	0.028	0.0797	761.6537	9.3103	0.9521	0.1539	0.2134	805.7097
5	10.8606	1.1078	0.2401	1.8238	754.7244	9.7225	1.0498	0.048	0.1211	730.1031	11.576	1.2002	0.5667	1.6066	779.3457
34	11.8237	1.1074	0.3386	2.2123	882.132	10.4165	1.0384	0.0707	0.1142	855.9576	12.848	1.2176	0.7921	2.0214	908.3065
107	5.0888	0.945	0.2435	1.1988	19.9419	4.6152	0.8865	0.0508	0.4296	17.9797	5.3589	1.0384	0.5698	0.6081	21.9041
146	9.6824	0.9767	0.3987	1.635	762.2101	8.4523	0.9017	0.0868	0.3712	747.0588	10.6404	1.0968	0.9244	1.1677	777.3614
126	8.6158	0.8212	0.0986	2.7716	860.2748	8.5914	0.8596	0.0328	0.6122	850.272	7.7757	0.7587	0.204	1.7154	870.2777
15	10.3139	0.9896	0.3399	1.3674	841.9368	9.0583	0.9204	0.0727	0.2592	824.8327	11.2546	1.1003	0.7909	1.052	859.041
169	7.612	0.7958	0.2614	4.1461	707.413	6.7069	0.735	0.0568	2.1225	704.162	8.2817	0.893	0.6061	0.9033	710.664
142	10.0024	0.9133	0.0911	0.9234	927.337	9.8619	0.9504	0.0315	0.1039	910.1736	9.1787	0.853	0.1863	0.7473	944.5004
60	12.0495	1.0839	0.0829	0.8544	952.1165	11.6954	1.1194	0.0305	0.4498	921.982	11.309	1.0259	0.1656	0.1831	982.251
202	10.547	1.0626	0.0797	0.6105	778.7547	10.3061	1.0975	0.0294	0.3089	753.0577	9.825	1.0058	0.1593	0.1602	804.4518
11	12.053	1.0839	0.0829	0.8544	952.5527	11.6986	1.1194	0.0305	0.4498	922.411	11.3124	1.0259	0.1656	0.1831	982.6943
124	10.9358	1.084	0.0853	0.8658	803.771	10.6715	1.12	0.0313	0.4551	776.131	10.1996	1.0253	0.1707	0.1873	831.411

Table 2 (A): Stability parameters estimates for thousand grain weight and grain yield for thirty DH lines, under Timely sowing (NOV), and late sowing (DEC) and Very late sowing (JAN) conditions.

	GY (Nov)					GY (Dec)					GY (Jan)				
GEN	**CV(%)**	**bi**	**DJi**	**Wi**	**Pi**	**CV(%)**	**bi**	**DJi**	**Wi**	**Pi**	**CV(%)**	**bi**	**DJi**	**Wi**	**Pi**
201	46.188	1.2063	37.7523	518.3948	4112.656	50.6954	1.2036	77.3223	256.6953	3976.682	52.5264	1.2093	73.4762	261.4551	4248.63
170	40.4062	1.1203	18.1574	197.5651	3500.473	44.3291	1.1146	36.3536	93.1671	3381	45.9714	1.1265	35.7464	104.3969	3619.947
195	38.2856	1.1139	8.7555	146.9994	3065.973	42.2077	1.1149	18.9922	76.1415	2942.067	43.3263	1.1127	15.9999	70.4758	3189.88
65	29.8995	0.9929	0.7981	3.628	1858.385	32.9952	0.9929	1.0713	1.291	1756.335	33.7838	0.9924	1.7804	2.0314	1960.436
145	31.1841	1.1073	6.8284	126.7796	1395.352	34.4103	1.1057	15.5986	63.9532	1330.833	35.2579	1.1089	11.6919	62.5945	1459.87
125	27.6403	1.0036	1.3256	5.4133	1188.184	30.6393	1.0051	2.0643	2.1761	1128.54	31.1116	1.0022	3.2032	3.224	1247.828
35	26.9968	1.0222	0.0461	4.4286	919.6781	29.9189	1.0228	0.1232	2.3804	876.1667	30.3973	1.0217	0.001	2.0268	963.1896
164	25.4364	0.9973	1.0115	4.1111	703.0272	28.1812	0.9984	1.4826	1.4934	660.56	28.6337	0.9958	2.4518	2.5285	745.4944
18	24.9292	1.0035	0.4258	1.8089	567.2298	27.5967	1.0043	0.3865	0.4681	531.0733	28.0771	1.0021	0.932	0.9501	603.3864
22	24.1189	1.0113	0.5591	3.3418	389.599	26.7424	1.0121	0.7698	1.4047	374.1683	27.1355	1.0104	1.4551	1.9225	405.0297
113	24.4663	1.0307	0.0389	8.3131	380.6628	27.1502	1.0317	0.0035	4.3574	372.0067	27.5116	1.03	0.0762	3.9377	389.3189
104	19.6624	0.7953	242.1905	1330.691	212.5573	17.3421	0.5953	363.2955	1071.904	274.5945	25.0385	1.0118	1.5354	2.132	150.5201
3	15.6043	-0.1195	664.1124	13477.89	1586.144	17.181	-0.1193	1320.46	6739.922	1444.167	17.5808	-0.1248	1309.379	6737.785	1728.12
94	36.3	1.04	0.7005	16.6083	3176.092	40.1213	1.0413	1.752	9.146	3061.607	40.9877	1.0389	0.9397	7.4417	3290.578
155	33.6215	1.0211	0.4105	5.4712	2639.016	37.0401	1.0215	0.307	2.3091	2506.44	38.0501	1.0199	0.789	2.4847	2771.591
102	32.5287	1.0007	0.0912	0.3686	2512.144	35.909	1.0017	0.0036	0.0157	2393.12	36.7485	0.9992	0.1645	0.167	2631.169
168	33.0432	0.9807	0.476	5.1038	2822.839	36.4426	0.9818	0.4486	1.8864	2687.802	37.357	0.9791	1.0321	2.9074	2957.876
149	35.6999	1.0945	5.2638	98.1023	2586.133	39.4259	1.0954	11.684	51.0874	2482.835	40.3359	1.0936	9.353	46.9281	2689.432
5	31.835	1.0498	0.1818	22.1128	1946.079	34.9421	1.0443	0.1897	8.6816	1844.5	36.1737	1.0549	0.1413	13.0681	2047.658
34	32.6375	1.0187	0.1475	3.6088	2393.956	35.7635	1.0125	0.0098	0.6838	2272.833	37.1427	1.0245	0.0138	2.5828	2515.078
107	27.6841	1.0392	0.0849	13.6247	984.0015	30.499	1.0339	0.0002	4.985	935.5	31.3578	1.0448	0.0036	8.6257	1032.503
146	32.8536	1.0426	0.1052	16.1137	2264.225	36.0704	1.0362	0.0076	5.6659	2165.373	37.3389	1.0493	0.0067	10.4478	2363.076
126	32.7668	1.0042	0.094	0.531	2546.27	36.3001	1.0058	0.0145	0.161	2453.993	36.9182	1.0032	0.0514	0.0947	2638.546
15	31.9958	0.9956	0.5117	2.2137	2408.632	35.1167	0.9895	0.8565	1.3297	2298.24	36.3681	1.0016	0.8686	0.88	2519.025
169	30.6556	1.0096	0.4392	2.5484	1933.664	33.784	1.0043	0.3865	0.4681	1860.973	34.7273	1.0157	0.4174	1.4691	2006.354
142	34.5323	1.0067	0.8865	3.9284	3001.58	38.1729	1.008	1.2969	1.5768	2884.708	38.9804	1.0054	2.2124	2.3374	3118.452
60	34.0685	1.0026	0.461	1.9041	2911.994	37.4866	1.0021	0.301	0.3205	2770.167	38.6034	1.0024	1.0038	1.0292	3053.822
202	35.5419	1.108	15.4625	162.6233	2430.092	39.2321	1.1089	32.9619	84.301	2328.846	40.1715	1.107	28.8734	78.0324	2531.339
11	34.5407	1.0114	0.1435	1.7045	2962.332	38.077	1.0125	0.0098	0.6838	2824.833	39.0712	1.0098	0.2059	0.6197	3099.83
124	30.9865	1.0109	0.2357	1.9684	2013.745	34.1514	1.0104	0.0357	0.5045	1903.507	35.0532	1.0107	0.4161	0.9076	2123.984

Table 2 (B): Stability parameters estimates for thousand grain weight and grain yield for thirty DH lines, under Timely sowing (NOV), and late sowing (DEC) and Very late sowing(JAN) conditions.

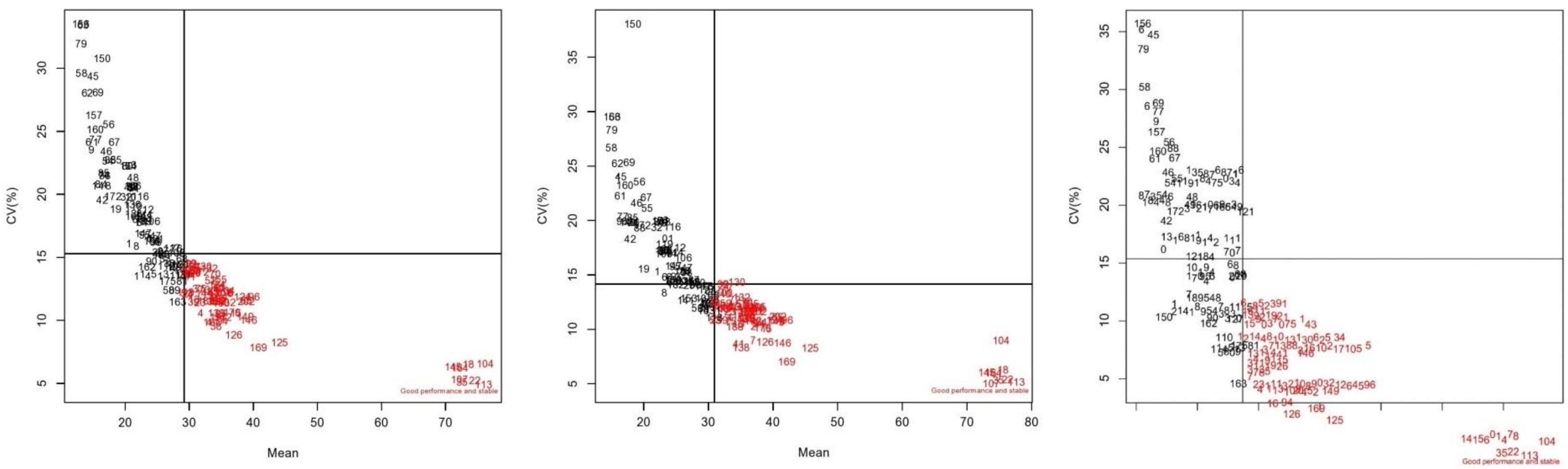


Fig 2: Stability analysis of thousand grain weight

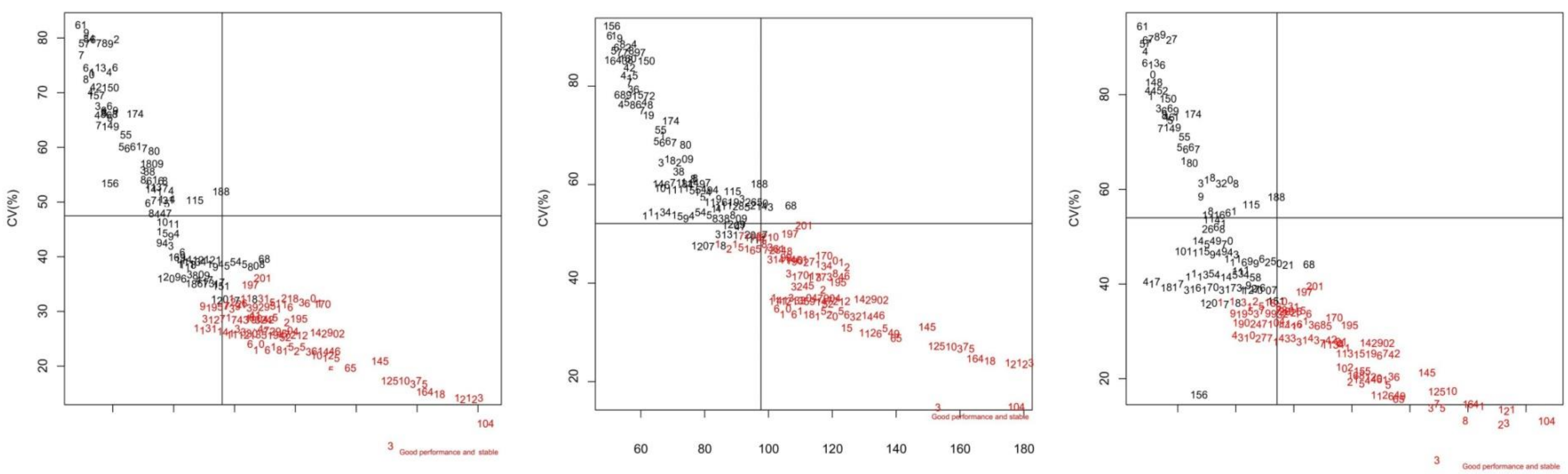


Fig 3: Stability analysis of grain yield

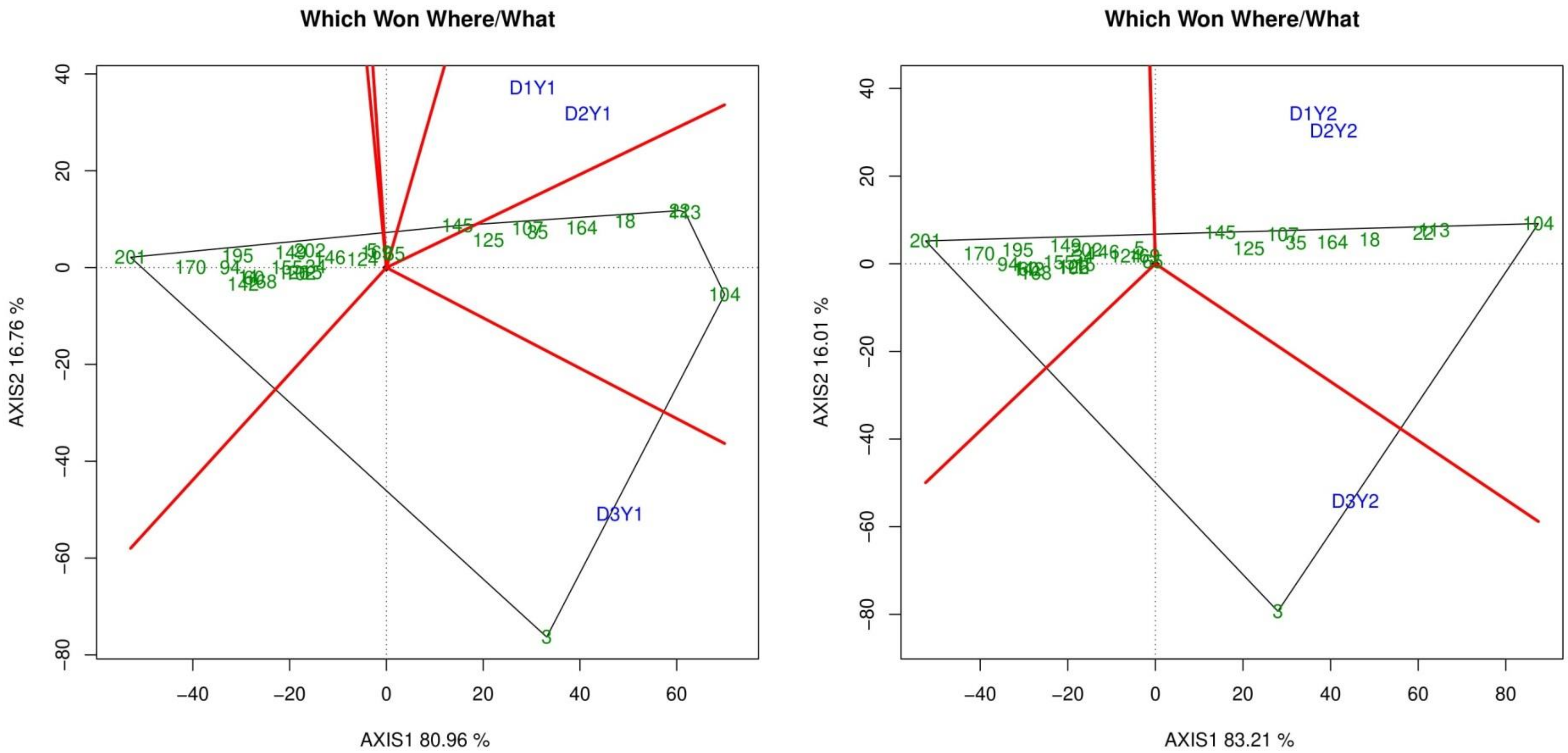


Fig 4: The polygon (which-won-where) view of genotype main effects plus genotype ×environment interaction effect (GGE) biplot of thousand grain weight of 30 DH lines tested in 2 years, over 3 different dates of sowing.

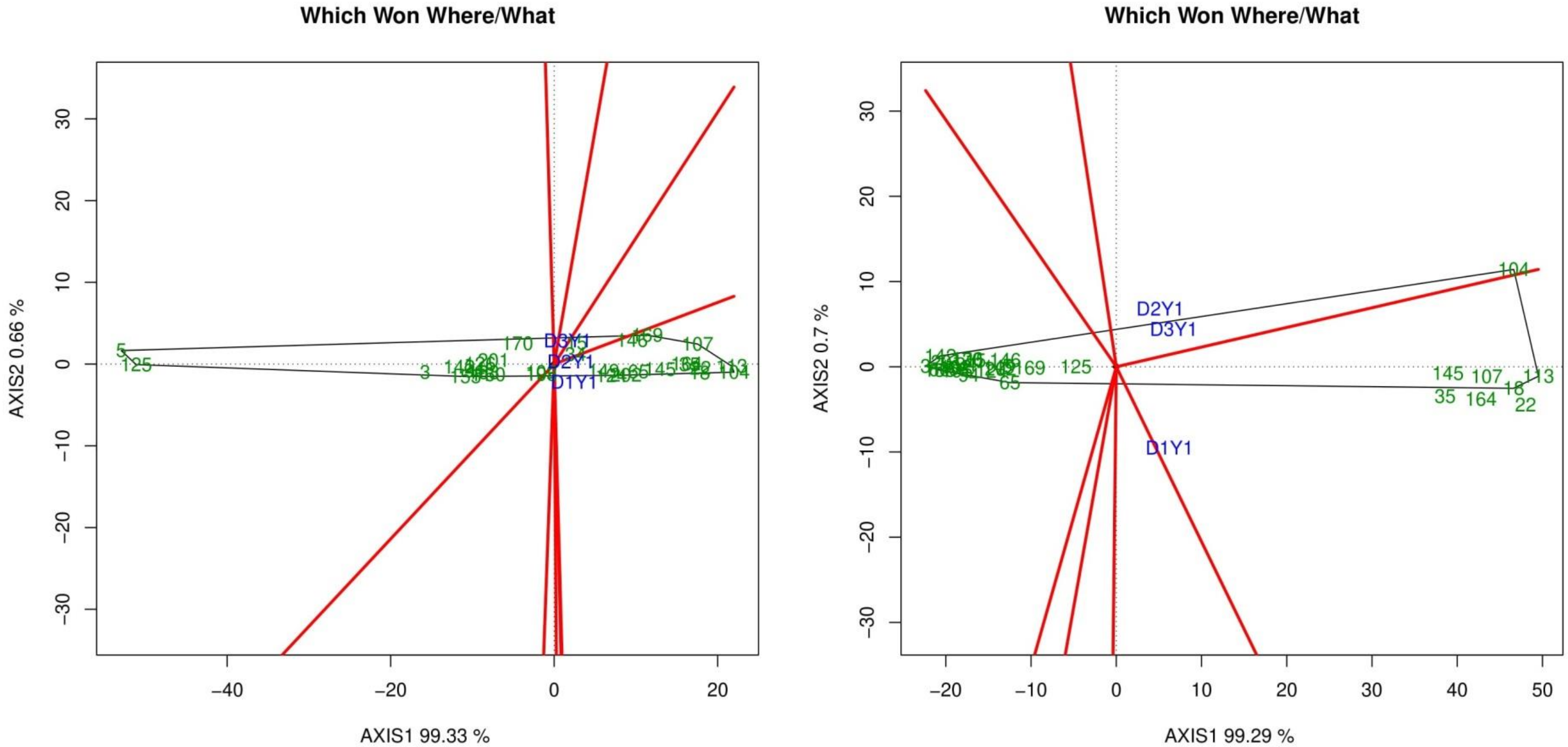


Fig 5: The polygon (which-won-where) view of genotype main effects plus genotype ×environment interaction effect (GGE) biplot of grain yield of 30 DH lines tested in 2 years, over 3 different dates of sowing.

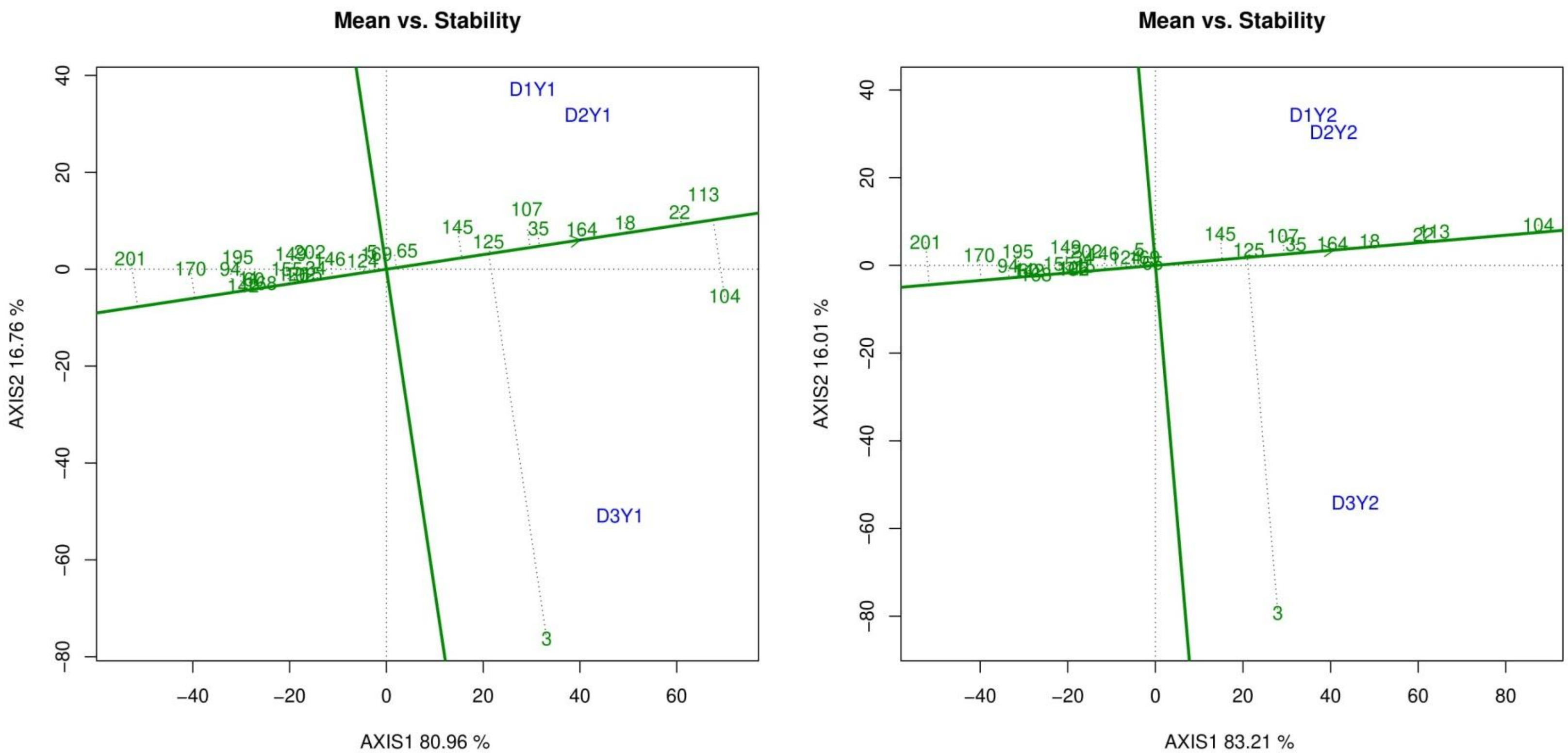


Fig 6: The mean vs. stability view of main effects plus genotype × environment interaction effect (GGE) biplot of thousand grain weight of 30 DH lines tested in tested in 2 years, over 3 different dates of sowing.

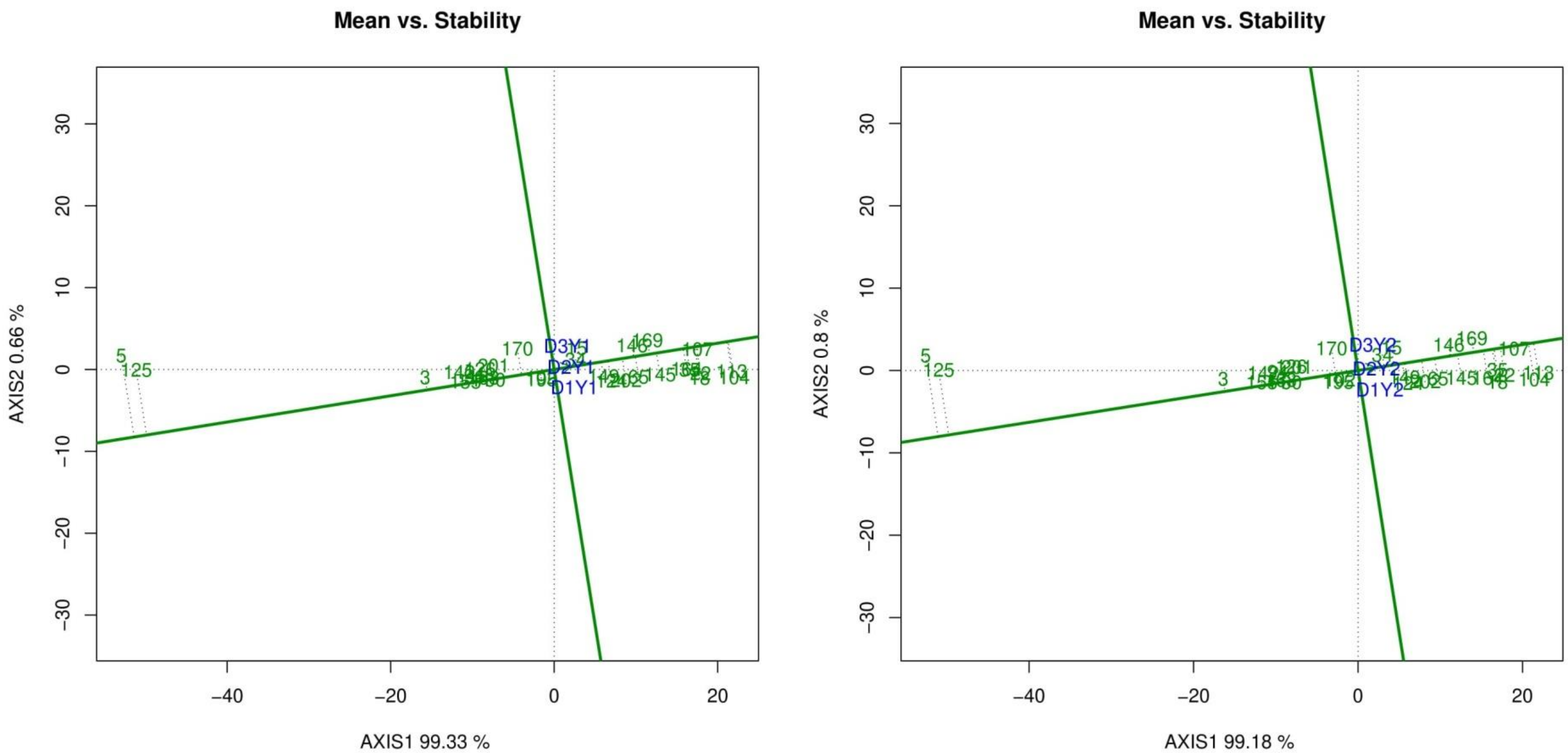


Fig 7: The mean vs. stability view of main effects plus genotype × environment interaction effect (GGE) biplot of grain yield of 30 DH lines tested in tested in 2 years, over 3 different dates of sowing.